injection with recombinant plasmid solution. In comparison with the rats pretreated with Ringer's solution or vector plasmid, those pretreated with recombinant plasmid had a lower mortality and lower serum transaminase levels. The injection with recombinant plasmid significantly reduced hemorrhage necrosis and inflammatory infiltration in the liver. Liver apoptotic index (AI) was dramatically lower in rats pretreated with recombinant vectors compared to the rats pretreated with Ringer's solution or vector plasmids. [(10.2±6.9)% vs. (83.1±12.6)% and (79.9±13.4)% respectively, $P < 0.01$]. In addition, the expression of exogenous Pim-3 gene remarkable inhibited the expression and secretion of TNF-α and IL-1β ($P < 0.01$).
Conclusions: Pim-3 gene can protect rats from LPS/D-GalN-induced fulminant hepatic failure possibly by inhibiting expression and secretion of inflammatory cytokines, such as TNF-α and IL-1β, in liver tissues.

PP-047 Method to isolate Kupffer cells from rats

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Background: Kupffer cells are one of the most important mesenchymal cells in liver, which play a crucial role in liver homeostasis. We report in detail a reliable and reproducible methodology for the isolation of KC, which gives highly purified and functionally intact cell cultures.
Methods: Firstly, a 20G catheter was inserted into rat's portal vein and secured with silk stitch, and then the liver was perfused and digested with DHANKS and HANKS containing 0.05% collagenase IV respectively; secondly, the Kupffer cells isolated by percoll gradient; and last step, isolated Kupffer cells were purified by selective adherence after 2 hours of cultivation. The kupffer cell identified by monoclonal antibody ED-2 and ED1. At same time, GFAP as a control antibody for telling apart HSC (T6 cell line) and Kupffer cells.
Results: This method resulted in a satisfactorily high yield of $6–18\times10^6$ KCs per liver, over 95% positive for ED-2 and ED1, negative for GFAP (Figures 1–3).
Conclusion: The method for isolating and culturing kupffer cells in this study is effective and stable, and the biological characters are preserved for further research.

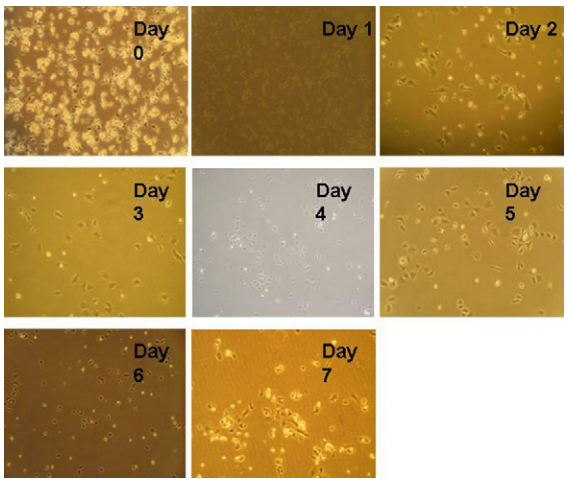

Figure 1. Kuppfer cells were cultured with density of 1×10^6/mL/well, 20% DMEM (high glucose), 10×.

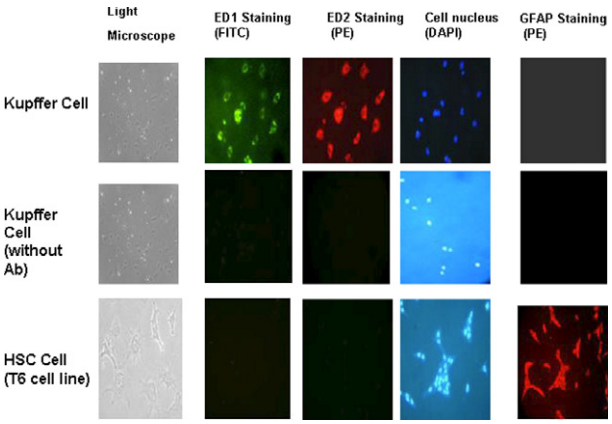

Figure 2. Immunofluorescence picture of Kuppfer cells and HSC cells.

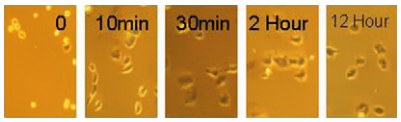

Figure 3. India ink staining of Kuppfer cells 10×.

PP-048 Mechanism of curcumine on the suppression of the expression of TGFβ1 and CTGF signaling pathway on experimental hepatic fibrosis

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Objective: Liver fibrosis is the common pathological basis for diverse liver injuries. Suppression of HSC activation and prevention of hepatic fibrogenesis attract many researchers' attention. Curcumin is used for anti-oxidant, anti-tumor, anti-inflammatory effects. However, its protective mechanism on hepatic fibrosis is not clear. The aim of the present study was to determine the mechanism of curcumine on the transforming growth factor beta1 (TGF-β1) and connective tissue growth factor (CTGF) pathway of hepatic fibrosis mice.
Methods: C57BL6/J mice were injected with CCL_4 for 8 weeks to induce hepatic fibrosis, and curcumine was given in the treated group. The effect of curcumine was assessed by comparing the severity of hepatic fibrosis in liver sections and the expression of TGF-β1, CTGF and smads.
Results: CCL_4 induced hepatic fibrosis models showed increased serum ALT and HA levels, infiltration of inflammation cells and fibrosis, which associated with enhanced α-SMA, TGFβ1, CTGF, TGFβRII and Smad3 protein expression and up-regulated TGFβ1, CTGF and Smad3 mRNA expression. The Smad7 mRNA and protein expression was down-regulated. Curcumine treatment significantly decrease serum ALT and HA levels and reduce hepatic fibrosis by repressing TGFβ1, TGFβRII, CTGF, Smad3 protein expression. It also repressing TGFβ1, CTGF, Smad3 mRNA expression. In addition, curcumine also increase smad7 mRNA and protein expression.
Conclusions: Curcumine may ameliorate hepatic fibrosis by inhibitingα-SMA activation and inhibiting TGFβ1 and CTGF signaling pathway. It shoud be a useful medicine for improving hepatic fibrosis.